PCR Amplification using Q5® High-Fidelity 2X Master Mix

Aim

Selectively amplify regions of interest from a linear or circular DNA molecule.

Procedure

1. Use of high quality, purified DNA templates greatly enhances the success of PCR. Recommended amounts of DNA template for a 50 μl reaction are as follows:

Table 1: Amount of DNA template

DNA	AMOUNT
DNA genomic	1ng - 1μl
Plasmid or viral	1pg - 1ng

2. Gently mix the reaction mix described below. Collect all liquid to the bottom of the tube by a quick spin if necessary.

Table 2: Reaction Setup

Component	**25 μl Reaction**	**50 μl Reaction**	**Final concentration**
Q5 High-Fidelity master mix	12.5 μl	25 μl	1x
10 μM Forward Primer	1.25 μl	2.5 μl	0.5 μM
10 μM Reverse Primer	1.25 μl	2.5 μl	0.5 μM
Template DNA	Variable	Variable	<1.000 ng
Nuclease-Free Water	to 25 μl	To 50 μl	

Table 3: Thermocycling Conditions for a Routine PCR

STEP	TEMP	TIME
Initial Denaturation	98°C	30 sec
25-30 Cycles	98°C	5-10 sec
	50-72°C	10-30 sec
	72°C	20-30 sec
Final Extension	72°C	2 min
Hold	4-10°C	

3. Transfer PCR tubes to a PCR machine and begin thermocycling.

Sources

To access the original protocol, click here.